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Emergence and control of an outbreak of infections due to Panton-Valentine leukocidin positive, ST22 methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit

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Abstract

Methicillin resistant *Staphylococcus aureus* (MRSA) infection can cause significant morbidity and mortality in neonates. We investigated a nosocomial MRSA outbreak in a neonatal intensive care unit (NICU), using a novel typing method. Following two fatal cases, in May 2011, a prospective outbreak investigation was conducted, involving neonates, mothers and healthcare workers in a large tertiary NICU in Sydney. MRSA isolates were characterized by antimicrobial susceptibility testing, a multiplex PCR-based reverse line blot (mPCR/RLB) binary typing system and other molecular typing methods. Over 7 months, 14 neonates were colonized with MRSA and six infected: three with superficial lesions and three with life-threatening disease, including the two index cases, who died despite empirical treatment with vancomycin. Isolates from 15 neonates were indistinguishable by RLB typing and identified as a PVL-producing ST22 SCC*mec* IV MRSA strain, which was resistant to gentamicin and trimethoprim-sulphamethoxazole. The outbreak strain was also isolated from one healthcare worker, one environmental swab and one father, but the source remained obscure. During the same period several different non-multiresistant and multiresistant MRSA strains were isolated from five neonates, five mothers (including two whose infants were colonized with the outbreak strain), one father, three healthcare workers and two environmental swabs. Rapid turnaround time of typing results allowed us to recognize and define the outbreak and implement targeted infection control interventions. PVL-producing ST22 SCC*mec* IV MRSA appears to be a virulent and highly transmissible pathogen in the NICU, which was difficult to control.

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This is the first description of an outbreak due to a rare clone of MRSA using a novel typing system. This highly discriminatory method is inexpensive, high throughput, has a turnaround time of 12 h, and enabled targeted infection control interventions.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has the potential to cause severe disease and sustained nosocomial outbreaks [1–3] among premature infants in neonatal intensive care units (NICUs). Its early recognition is essential to prevent transmission and ensure optimal treatment [4].

The mother is often assumed to be the source of MRSA in a colonized neonate, but nosocomial transmission from fomites or via healthcare workers (HCWs) also occurs. Transmission routes can be identified with the aid of molecular typing [3].

In May 2011, two extremely premature (26 weeks gestation) infants developed fulminant MRSA sepsis and died within a few days of each other. This paper describes the outbreak investigation, using a novel rapid MRSA strain-typing method.

Methods

Design

This was a prospective study in which infection control interventions were based upon molecular typing data available in real time.

Setting

Westmead Hospital is a 980-bed teaching hospital in Sydney, Australia, serving a population of 1.5 million; there are *c.* 5500 deliveries annually. The 39-bed NICU comprises 19 high acuity ventilator cots and 20 lower acuity special care cots; in 2011 there were 1635 admissions, including over 100 infants <1500 grams birth weight; 1066 patients were admitted during the outbreak period (18 May 2011 to 23 January 2012). The standard nurse/bed ratio is 1:1–1:4, depending upon staffing levels and patient acuity. There are no single cot isolation rooms.

Patients and case definition

Outbreak cases were defined by isolation of the MRSA outbreak strain from any culture during the outbreak period. In these infants, infection was defined by clinical and laboratory criteria and a requirement for antimicrobial therapy, and colonization by absence of relevant symptoms.

Interventions

MRSA surveillance. . Previously, there had been no routine MRSA screening in the NICU because MRSA infections were rare. After two index cases were identified (Fig. 1), all neonates and NICU staff were screened and, subsequently, neonates were swabbed on admission and weekly thereafter. Because of ongoing MRSA acquisitions, NICU staff screening was repeated and extended to delivery room staff in September 2011. Swabs were collected from the anterior nares, umbilicus (if moist) and perineum of neonates and anterior nares, only, of staff. Mothers of colonized or infected neonates were screened using nose, throat, perineal and low vaginal swabs. Frequently touched environmental surfaces, including door handles, trolleys, computer keyboards, ultrasound probes and arterial blood gas and X ray machines, were swabbed in May and September 2011, using sterile, dry swabs, which were inoculated directly into broth (see below).

Decolonization regimen. . MRSA-colonized HCWs were treated with mupirocin 2% nasal ointment three times a day and daily triclosan 1% body wash for 5 days.

Culture and strain typing

Surveillance swabs from individual infants and mothers were inoculated, together, into a single tube of methicillin, aztreonam, mannitol salt (MAMS) broth. Environmental swabs were inoculated directly into MAMS broth. Broths were incubated for 18 to 24 h at 35°C and subcultured onto chromogenic Brilliance™ MRSA selective media (Oxoid, Basingstoke, Hants, UK). Molecular methods were also employed for rapid detection, using the MRSA4 Easy-Plex assay kit (AusDiagnostics, Sydney, Australia), which targeted nuc, mecA and SCCmec-orfX; all positive samples were confirmed by culture. Pure cultures were stored in nutrient broth with 20% glycerol at −70°C. Isolates were identified by routine laboratory methods [5], including antibiotic susceptibility testing performed by BD Phoenix Automated Microbiology System (Becton Dickinson, Sparks, MD, USA).

Molecular typing studies

Isolates were typed using a novel binary typing system for MRSA (O'Sullivan MV, Sintchenko V, Gilbert GL; personal communication). The 19 targets for this assay were chosen from 51 utilized in previous assays for toxin gene [6], phage-derived open reading frame [7] and SCC*mec* typing [8]. Results of these assays, using a diverse local and international collection of 165 MRSA isolates, were analysed by a specially designed computerized algorithm (AuSeTTS, available at

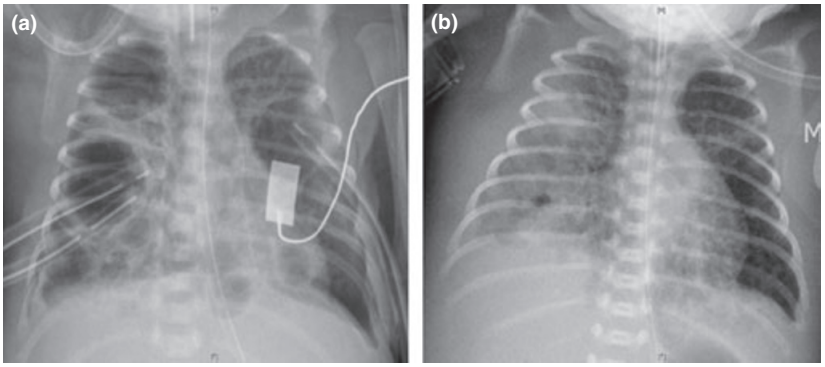

FIG. 1. Chest radiographs of Cases 1 and 2. (a) Case 1: rapidly progressive necrotizing pneumonia. (b) Case 2: overwhelming sepsis.

http://www.cidmph.org.au/pages/AuSeTTS) (O'Sullivan MV, Sintchenko V, Gilbert GL; submitted for publication) to identify the combination of targets with the highest discriminatory power, while maintaining concordance with multilocus sequence typing (MLST). Targets selected were four toxin genes (*sea*, *sec*, *sed* and *lukS-PV*), nine derived from integrated prophages (Tn554*tnpB*, φMu50B SAV0881, φPV83 ORF 2, φMu50B SAV0858, φ11 nt 4427-5251, φSLT ORF 257, φN315 SA1801, φMu50A SAV1974 and φSLT ORF 182) and six SCC*mec* elements (*ccrAB*, *ccrC*, *mecR1*, E007, CQ002 and *cadB*) plus nuc (*S. aureus* control) and mecA (MRSA control). All targets were amplified in a single multiplex PCR reaction; products were hybridized to probes on a reusable nylon membrane and detected by chemiluminesence, visualized on X-ray film [9]. Results were expressed as a 19-digit binary number, converted to a decimal code for ease of interpretation. The method's discriminatory power (Simpson's index of diversity, D = 0.994; 95% CI, 0.988–1.00) was similar to that of PFGE (D = 0.987; 95% CI, 0.977–0.998) and higher than that of *spa* typing (D = 0.926; 95% CI, 0.879–0.972) and its concordance with MLST was high (adjusted Wallace coefficient 0.993; 95% CI, 0.986–1.000 [10]). The assay was performed weekly and, during the outbreak investigation, selected isolates from the NICU were further characterized.

Pulse field gel electrophoresis (PFGE) was performed according to the harmony protocol [11]; patterns were examined using BIONUMERICS v3.00 software (Applied Maths, Sint-Martens-Latem, Belgium). PFGE types were defined by indistinguishable patterns (100% similarity). *spa* typing [6] and MLST [12] were performed according to previously published methods. SCC*mec* subtyping was performed using mPCR/RLB assay [8]; subtypes were assigned as proposed by Chongtrakool *et al.* [13].

Results

Between 18 May 2011 and 23 January 2012, 20 MRSA colonized or infected neonates (including three sets of twins) were identified in the NICU (Fig. 2). Fifteen were outbreak cases, of whom four had MRSA infection: one each with purulent ear discharge and severe pneumonia and the two index cases, with fatal sepsis.

Case 1 had rapidly progressive sepsis and necrotizing pneumonia, with pneumatocoeles. MRSA was isolated from tracheal aspirate and urine but not blood. Case 2 developed overwhelming sepsis, with MRSA in blood and CSF, 2 days after MRSA had been isolated from a nasal lesion. Both infants were treated empirically with vancomycin at the onset of symptoms and later with linezolid and/or clindamycin. Clinical presentations and outcomes of all 20 cases (see below) are summarized in Table 1.

In May 2011, surveillance cultures were collected from 138 NICU HCW; two (staff A and B) were colonized and successfully decolonized. In June 2011, a bundle of enhanced infection control measures was implemented: MRSA-

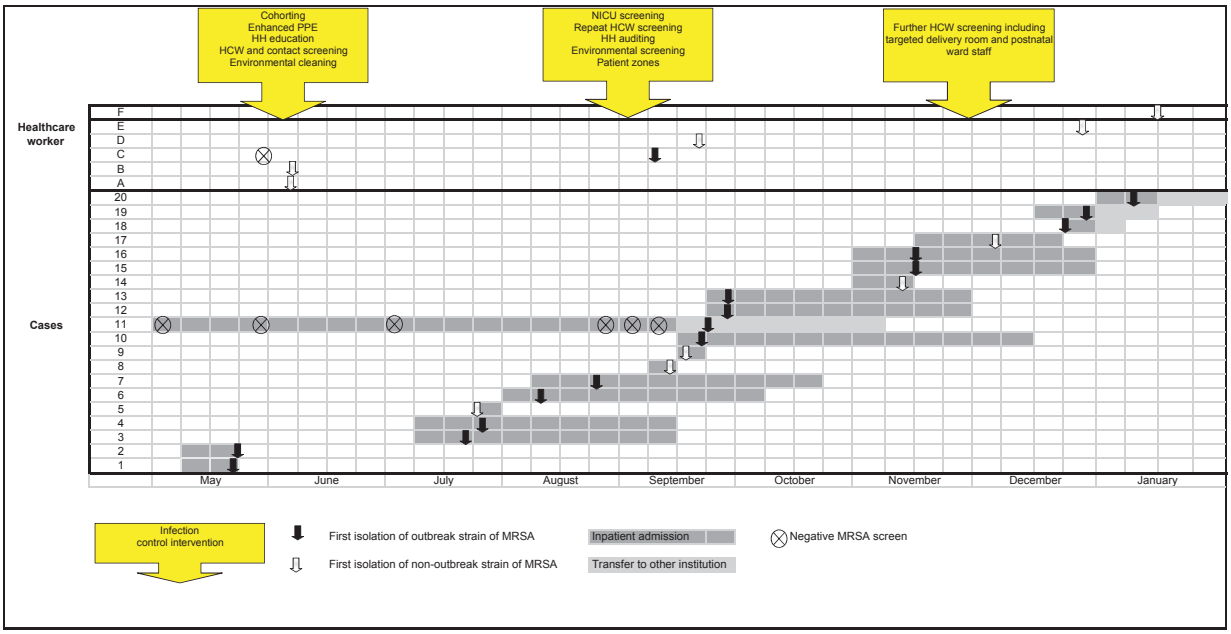

FIG. 2. Outbreak timeline between 18 May 2011 until 23 January 2012. PPE, personal protective equipment; HH, hand hygiene; HCW, healthcare worker.

TABLE 1. Clinical data for infants colonized or infected with methicillin-resistant *Staphylococcus aureus* (MRSA)

CASE	Sex/ethnicity	Gestational age, weeks	Birth weight, g	Birth	Underlying conditions (s)	Age in days at first isolation of MRSA	Risk factors	Clinical presentation	Infection/ colonisation	Other manifestations	Site	Antimicrobial treatment	Outcome
1	F/Caucasian	26	730	LSCS	HMD, PDA, IUGR, XXX anomaly, Grade 1 IVH	9	UVC, CVC, ICC, TPN	Septic shock	Infection	Pneumothorax	ETT aspirate	Penicillin, gentamicin, vancomycin, cefotaxime, meropenem	Died
2	F/East or SE Asian	26	830	PROM, Vaginal breech	HMD, PDA, Grade 1 IVH	11	UVC, UAC, CVC, ICC	Septic shock, chest infection	Infection	Pneumothorax, anaemia of prematurity	Blood	Penicillin, gentamicin, meropenem, vancomycin	Died
3	F/Middle Eastern	28	1010	PROM, LSCS	HMD, ROP	2	CVC	Suspected sepsis	Colonisation	GORD	Surveillance swab	Penicillin, gentamicin, vancomycin	Survived
4	M/Middle Eastern	28	1310	PROM, LSCS	HMD, PDA, Grade 2 IVH	8	CVC	Suspected sepsis	Colonisation	GORD	Surveillance swab	Penicillin, gentamicin, vancomycin	Survived
5	M/Caucasian	40	4122	PROM, LSCS	None	8	PVC	R breast abscess	Infection	None	Umbilicus	Flucloxacillin, gentamicin, vancomycin	Survived
6	M/Caucasian	27	1210	LSCS	HMD, PDA	4	PVC	L purulent discharge from ear	Infection	CLD, GORD	Ear	Penicillin, gentamicin, vancomycin	Survived
7	M/Caucasian	25	780	LSCS	HMD, PDA, ROP	11	UVC, UAC, CVC	Sepsis, seizures, pneumonia	Infection	GORD, conjugated hyperbilirubinaemia	Eye	Penicillin, gentamicin, vancomycin, rifampicin, clindamycin, meropenem	Survived
8	M/South Asian	38	3100	PROM, NVB	None	2	PVC	Pustules on head	infection	None	Surveillance swab	Penicillin, gentamicin, flucloxacillin	Survived
9	M/Caucasian	38	4040	elective LSCS	None	3	None	Haematuria	colonisation	None	Bag urine	None	Survived
10	F/Caucasian	26	1000	LSCS	HMD, NEC, Grade 1 IVH	5	UVC, CVC	NEC	Colonisation	None	Surveillance swab	Penicillin, gentamicin, vancomycin, clindamycin	Survived
11	M/Caucasian	26	890	LSCS	HMD, PDA, ROP	141	UVC, UAC, CVC	Suspected sepsis	Colonisation	CLD, metabolic bone disease, bilateral inguinal hernias	Surveillance swab	Penicillin, gentamicin, cefotaxime, vancomycin, augmentin	Survived
12	F/Middle Eastern	27	1100	LSCS	HMD	1	CVC	Coagulopathy	Colonisation	GORD, PDA,	Surveillance swab	Penicillin, gentamicin	Survived
13	F/Middle Eastern	27	1210	LSCS	HMD, PDA	1	UVC, UAC, CVC	Suspected sepsis	Colonisation	PDA	Surveillance swab	Penicillin, gentamicin	Survived
14	F/Caucasian	40	3195	NVB		10	None	L breast	Colonisation	None	Surveillance swab	Vancomycin gentamicin,	Survived
15	M	32	1260	LSCS	HMD, PDA	15	UAC, UVC, CVC	Suspected sepsis	Colonisation	CLD, Bilateral inguinal hernia, penoscrotal hypospadius	Surveillance swab	vancomycin gentamicin,	Survived
16	M	32	1165	LSCS	HMD, PDA	15	UAC, UVC, CVC	Midgut volvulus	Colonisation	CLD, Bilateral inguinal hernia, penoscrotal hypospadius	Surveillance swab	Vancomycin gentamicin, Metronidazole	Survived
17	F	38	2640	Vaginal breech	Trisomy 21, AVSD	8	None	Heart failure	Colonisation	None	Surveillance swab	None	Survived
18	M/Middle Eastern	36 + 3	1820	NVB	HMD	1	PVC	Suspected sepsis	Colonisation	None	Surveillance swab	Penicillin, gentamicin	Survived
19	M/Middle Eastern	31 + 2	1890	NVB	HMD	9	PVC	Suspected sepsis	Colonisation	None	Surveillance swab	Penicillin, gentamicin	Survived
20	M/Caucasian	36 + 4	2420	LSCS	TTN	3	None	Asympatomatic	Colonisation	None	Surveillance swab	None	Survived

ASD, atrial septal defect; AVSD, atrioventricular septal defect; CLD, chronic lung disease; HMD, hyaline membrane disease; PDA, patent ductus arteriosus; GORD, gastroesophageal reflux disease; IVH, intraventricular haemorrhage; IUGR, intrauterine growth retardation; NEC, necrotising enterocolitis; NVB, normal vaginal birth; PROM, premature rupture of membranes; PVC, peripheral venous canula; ROP, retinitis of prematurity; UAC, umbilical arterial catheter, UVC, umbilical venous catheter; CVC, central venous catheter; ICC, intercostal catheter; TPN, total parenteral nutrition, TTN,Transient Tachypnoea of Newborn.
Cases that isolated the outbreak strain are highlighted.

colonized infants were placed on contact precautions and cohorted until discharge; and a unit-wide review of hand hygiene practices, staff education on '5 Moments of Hand Hygiene' (Fig. 2) and enhanced environmental cleaning of the MRSA-colonized patient rooms were implemented.

Despite these measures, there was ongoing MRSA acquisition, including by three infants (twins and a singleton) who were positive on admission to the NICU, following Caesarean section delivery. Therefore, a second bundle of measures was implemented in September 2011. Additional or repeat MRSA screening of NICU staff was extended to staff in the delivery suite. Two of 146 NICU HCW (20 doctors, 107 nurses and 19 patient support assistants, technicians and cleaning staff) screened were colonized (staff C and D; both were negative in June). Two of 83 delivery suite staff (26 obstetricians, 33 midwives, 23 anesthetic staff and one other) screened were colonized (staff E and F). All colonized staff were successfully decolonized.

Accredited auditors performed daily '5 moments' hand hygiene audits and compliance improved from 81% in September to 95% in January 2012; audits were subsequently performed weekly. Patient zones were physically demarcated to improve adherence to contact precautions. Additional environmental screening yielded MRSA from three of 52 swabs: a blood gas machine, a milk trolley and a curtain. Five of 13 mothers (38%; 95% CI 31–45%) and both of two fathers screened were MRSA colonized (see Table 2).

Molecular typing results

RLB binary typing identified 18 indistinguishable MRSA isolates (the 'outbreak' strain) from 15 neonates, one HCW (staff C), one father and one arterial blood gas machine swab (Table 2). This strain was PVL positive, ST 22, SCC*mec* subtype IV.3.1.2 (or IVc) and *spa* type t005. The PFGE patterns of these isolates were indistinguishable.

All colonized mothers carried non-outbreak strains, three of which matched those of their infants. The other two were mothers of outbreak cases (6 and 7). Staff C, who was colonized with the outbreak strain, had cared for outbreak cases 3 and 4. Staff D was colonized with the same non-outbreak strain as case 8, whom she had nursed. This multiresistant strain was identified as ST772-V, *spa* type t657 (Bengal Bay clone). Six non-outbreak isolates with indistinguishable RLB types were isolated from a midwife (Staff F), three babies (cases 5, 9 and 14), one mother (9) and a delivery suite curtain. Apart from the mother/baby pair (case 9), there were no apparent epidemiological links; staff F was not involved in delivery of those babies. These strains were typical of ST1-IV (USA400 or WA-MRSA-1), which is the second most common community-associated MRSA clone in Australia [14].

Discussion

We documented sustained nosocomial transmission of a virulent MRSA PVL-positive ST22-MRSA-IV in our NICU, affecting 15 neonates, one father and one HCW over a 7-month period. This is the first report of a nosocomial outbreak due to this clone in Australia. PVL, which is thought to be important in pathogenesis [15,16], probably contributed to fatal necrotizing pneumonia with pneumatocoele formation, in one index case, and to septic shock and bullous lung abscesses in the other.

We demonstrated the utility of our novel mPCR/RLB typing method, to rapidly identify nosocomial transmission and define the outbreak by excluding MRSA-colonized infants carrying non-outbreak strains. Traditional MRSA typing methods are too expensive, too slow (PFGE, MLST) and/or have insufficient discriminatory power (MLST) for routine use. *spa* typing has been used, prospectively, to identify nosocomial transmission events [17] but is unsuitable in our institution, where the dominant MRSA clone (ST239-III) belongs to a single *spa* type (t037). Our binary typing method has a turnaround time of 12 h and high discriminatory power; it is inexpensive (~US$2 per isolate) and up to 80 isolates can be typed in a single run, using two membranes. It allows targeted infection control interventions to be implemented promptly.

The index cases shared a room; it was not clear who was colonized first, but transmission between them, by HCW or fomites, was assumed. Their mothers were not screened; one may have acquired infection through vertical transmission or from an unidentified HCW. No common source for subsequent cases was found; it was unlikely to have been the HCW colonized with the outbreak strain, after a previously negative screen. The outbreak strain was isolated from only one of more than 50 environmental swabs, but demonstrates the potential for MRSA to be transmitted via fomites and the need for targeted environmental cleaning and enhanced hand hygiene. Twelve of 15 outbreak cases were delivered by Caesarean section, including three colonized within 24 h of birth, suggesting acquisition by some from a delivery suite source, as well as transmission within the NICU.

Further evidence favouring nosocomial, rather than vertical, transmission was that mothers of two outbreak cases were colonized with non-outbreak strains, whereas colonized mothers of babies with non-outbreak strains carried the corresponding strain. Several studies have suggested that maternal vaginal MRSA colonization leading to neonatal infection is uncommon [18–20].

TABLE 2. MRSA isolate information

Sample	Date	Antibiotic Resistances	PVL	MLST (Predicted)'	SCCmec (Predicted)'	*spa* (Predicted)'	Binary typing result	Probe results: sea	sec	sed	pvl	N046	SAV0881	PV830RF2	SAV0858	phi11-4568	sitORD257	SA1801	SAV1974	sitORF182	ccrAB1	ccrC	mecR1	E007	CQ002	cadB
Baby 1	18/05/2011	Gen TmpSmz	+	22	**IV.3.1.2**	*t005*	**37 248**																			
Baby 2	22/05/2011	Gen TmpSmz	+	22	**IV.3.1.2**	**t005**	**37 248**																			
Baby 3	28/07/2011	Gen TmpSmz	+	22	**IV.3.1.2**	*t005*	**37 248**																			
Baby 4	17/07/2011	Gen TmpSmz	+	22	*IV.3.1.2*	**t005**	**37 248**																			
Baby 5	28/07/2011	Nil	+	*1*	*IV*	**t127**	**308 034**																			
Baby 6	9/08/2011	Gen TmpSmz	+	22	**IV.3.1.2**	**t005**	**37 248**																			
Baby 7	23/08/2011	Gen TmpSmz	+	22	**IV.3.1.2**	*t005*	**37 248**																			
Baby 8	13/09/2011	Cip EryGen TmpSmz	+	*772*	**V**	*t657*	**427 152**																			
Baby 9	16/09/2011	Nil	+	*1*	*IV*	*t127*	**308 034**																			
Baby 10	19/09/2011	Gen TmpSmz	+	**22**	**IV.3.1.2**	**t005**	**37248**																			
Baby 11	21/09/2011	Gen TmpSmz	+	22	**IV.3.1.2**	**t005**	**37248**																			
Baby 12	28/09/2011	Gen TmpSmz	+	22	**IV.3.1.2**	*t005*	**37 248**																			
Baby 13	28/09/2011	Gen TmpSmz	+	22	**IV.3.1.2**	*t005*	**37 248**																			
Baby 14	9/11/2011	Nil	+	*1*	*IV*	*t127*	**308 034**																			
Baby 15	14/11/2011	Gen TmpSmz	+	22	**IV.3.1.2**	*t005*	**37 248**																			
Baby 16	14/11/2011	Gen TmpSmz	+	22	**IV.3.1.2**	*t005*	**37 248**																			
Baby 17	4/12/2011	Cip Ery	-	22	**IV**	*t022*	**79 744**																			
Baby 18	21/12/2011	Gen TmpSmz	+	22	*IV.3.1.2*	*t005*	**37 248**																			
Baby 19	26/12/2011	Gen TmpSmz	+	22	*IV.3.1.2*	*t005*	**37 248**																			
Baby 20	8/01/2012	Gen TmpSmz	+	22	*IV.3.1.2*	*t005*	**37 248**																			
Mother 6	11/08/2011	Cip Ery Clin Fos	-	*5*	**II.1.1.1**	*t002*	**152 393**																			
Mother 7	26/08/2011	Cip Ery	+	*8*	**IV**	*t024*	**37 762**																			
Mother 9	17/09/2011	Nil	+	*1*	*IV*	*t127*	**308 034**																			
Mother 17	9/12/2011	Cip Ery	-	22	*IV*	*t022*	**79 744**																			
Father 17	9/12/2011	Cip Ery	-	22	*IV*	*t022*	**79 744**																			
Father 20	11/01/2012	Gen TmpSmz	+	22	*IV.3.1.2*	*t005*	**37 248**																			
Mother (infant unaffected)	21/12/2011	Nil	-				**268 162**																			
Staff A (midwife)	6/06/2011	Cip Ery Clin Gen TmpSmz	-	*239*	**III**	*t037*	**279 833**																			
Staff B (medical officer)	6/06/2011	Cip Gen TmpSmz	+		**II**		**46 464**																			
Staff B (medical officer)	26/06/2011	Cip Ery Clin Gen	-	*239*	**III**	*t037*	**280 845**																			
Staff C (midwife)	12/09/2011	Gen TmpSmx	+	22	**IV**.3.1.2	*t005*	**37 248**																			
Staff D (midwife)	21/09/2011	Cip EryGen TmpSmz	+	**772**	*V*	**t657**	**427 152**																			
Staff E (medical officer)	25/12/2011	Cip Ery	-	22	*IV*	*t032*	**13 184**																			
Staff F (midwife)	23/01/2012	Nil	+	*1*	*IV*	*t127*	**308 034**																			
Blood gas machine	12/09/2011	Gen TmpSmz	+	22	**IV.3.1.2**	*t005*	**37 248**																			
Milk Trolley	12/01/2012	Nil	-				**65 794**																			
curtain	20/01/2012	Nil	+	*1*	*IV*	*t127*	**308 034**																			

Antibiotic resistances are for non-beta lactam antibiotics only; antibiotics tested were chloramphenicol, ciprofloxacin (Cip), clindamycin (Clin),erythromycin (Ery), fosfomycin (Fos), fusidic acid, gentamicin (Gen), linezolid, mupirocin, nitrofurantoin, quinupristin-dalfopristin, rifampin and trimethoprim-sulphamethoxazole (TmpSmz). Each target in the binary typing system was interrogated using two probes (sense and antisense), black squares indicated positive probes. Isolates belonging to the outbreak strain are highlighted. MLST, SCC*mec* and *spa* results in bold represent actual results; those in italics are predicted based on the binary typing result.

Binary typing showed, and formal MLST, *spa* and SCC*mec* typing confirmed, that the outbreak strain was PVL positive and unrelated to the usual nosocomial clones seen in our institution (AUS2/3 [ST239 MRSA-III] and UK-EMRSA15 [ST22-MRSA IV]). PFGE confirmed that the outbreak isolates were indistinguishable. PVL-positive ST22 MRSA has been rarely identified, previously, in Australia [14].

ST22-MRSA-IV is generally synonymous with UK-EMRSA-15, which emerged in hospitals in the United Kingdom in 1991 [21], was subsequently identified in Germany where it has become the predominant MRSA clone [22] and has spread to hospitals and long-term care facilities, worldwide. It is characteristically ciprofloxacin resistant and gentamicin susceptible, may produce enterotoxin C but rarely PVL, harbours SCC*mec* subtype IVh and has *spa* type t022 or t032 (or a related variant) [23].

Our outbreak strain differs from EMRSA-15; it has a different SCC*mec* subtype (IV3.1.2 or IVc), *spa* type (t005), antibiogram (resistant to gentamicin and trimethoprim-sulphamethoxazole but susceptible to ciprofloxacin) and produces PVL, but not enterotoxin C. It has been reported in the United Kingdom [24,25] and Japan [26], where the index case reported recent travel to India. An outbreak of breast abscesses in postpartum women in Mumbai, India, was due to a similar MRSA clone, differing only by a single nucleotide polymorphism in one *spa* repeat [27]. There was no known link with the Indian subcontinent in our cases, but staff and patients at our institution come from a broad range of ethnic backgrounds, so recent importation of this clone remains a possibility.

Considering the large number of differences in the non-core genome, this clone probably arose from ST22-MSSA, independently of EMRSA-15 [24]. An outbreak of PVL-positive ST-22 MSSA with the same *spa* type and antibiogram has been reported from Italy, also amongst postpartum women and neonates [28].

The methods we used for MRSA screening included both PCR and broth enrichment to optimize turn-around time and sensitivity, followed by subculture. Previous studies suggest that PCR-based screening has high sensitivity but low reproducibility in neonates and that a combined approach, with confirmatory culture, is preferable [29]. For optimal neonatal screening, the combination of umbilical and nasal swabs has a reported sensitivity of >90% [30]. The screening protocol we used (pooled ear, nose, umbilicus and perineum) should have similar sensitivity. However, only nasal swabs were collected from staff, which may explain the major limitation of this investigation, namely our failure to identify a source, despite extensive screening of those involved in delivery and care of these infants.

Among the mothers of colonized infants screened for MRSA, the carriage rate (of non-outbreak strains) was high (38%). Previous reports suggest overall anovaginal MRSA carriage rates of 2–4%, with low rates of vertical transmission of MRSA infection to neonates [18,19]. Systematic screening is required to determine the true MRSA carriage rates in our antenatal population but was beyond the scope of this study.

Non-multiresistant MRSA is an emerging pathogen in the NICU, and can cause outbreaks with significant mortality. The virulent PVL-positive ST22-MRSA-IV strain that caused this outbreak was difficult to control, despite timely use of a novel typing system, which allowed accurate definition of the outbreak, and implementation of targeted infection control interventions that have, apparently, finally terminated the outbreak. At the time of writing there have been no new acquisitions of the outbreak strain for more than 4 months. EMRSA-15 is not the only ST22-MRSA-IV clone with epidemic potential.

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Transparency Declaration

The authors declare no conflict of interests.

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